PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12302650/e	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/001077	International Filing Date *(day/month/year)* 22 August 2003	Priority Date *(day/month/year)* 23 August 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C07K 1/04, C12Q 1/68, G01N 33/53, G01N 33/543

Applicant

GENERA BIOSYSTEMS PTY LTD et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [X] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
2 March 2004	1 December 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer DAVID GRIFFITHS Telephone No. (02) 6283 2628

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001077

Basis of the report

With regard to the elements of the international application:*

☐ the international application as originally filed.

☒ the description, pages 1 - 35, as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages 36 - 41, received on 23 November 2004 with the letter of 23 November 2004

☒ the drawings, pages 1 - 5, as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims 1 - 30	YES
	Claims	NO
Inventive step (IS)	Claims 1 - 30	YES
	Claims	NO
Industrial applicability (IA)	Claims 1 - 30	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The present application relates to a method for producing a plurality of carriers for immobilised molecules, such as nucleic acid molecules, labelled with an identifiable code molecule that allows differentiation of labelled carriers from other labelled carriers in a heterogeneous population of such carriers. The method comprises:

(i) preparing a plurality of carriers having different code molecules each code associated with a respective carrier;

(ii) subjecting the nucleic acid molecules to nucleic acid-based reactions to enable incorporation of detectable labels into the immobilised nucleic acid molecules;

(iii) identifying carriers having distinctive code molecules that are detectably and/or quantifiably decipherable or resolvable by the detection/quantification means;

(iv) identifying carriers having similar to non-distinctive code molecules; and

(v) sorting carriers having distinctive code molecules from the carriers having non- distinctive code molecules to thereby provide a plurality of carriers including a population having detectably distinct code molecules.

The following citations are considered in this report:

D1. WO 2001/062772

D2. WO 2001/046460

D3. WO 1997/014028

D4. *Proc. Natl. Acad. Sci.* Vol 90, pp. 10700-10704, 1993

WO 2001/062772 discloses a method for synthesising, encoding and decoding compounds in a combinatorial library. The method includes a self-encoding step in which different pairs of components — each pair with a known, different molecular weight difference — are reacted with supports, so that two compounds differing in molecular weight are formed on each support. The citation does not disclose or suggest the step of sorting carriers having distinctive code molecules from those with non-distinctive code molecules to produce a plurality of carriers including a population having detectably different code molecules. Therefore the present claims must be considered novel and inventive over this citation.

WO 2001/046460 discloses a method for analysing nucleic acid sequences by hybridising nucleic acid fragments on complementary sequences immobilised on coded supports; hybridising probes onto these nucleic acid fragments then sequentially identifying the coded supports by mass spectrometer. The method allows the real-time analysis of DNA or RNA and highly parallelisable high-output sample preparation. The citation does not disclose or suggest the step of sorting carriers having distinctive code molecules from those with non-distinctive code molecules and so the present claims must be considered novel and inventive over this citation.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

In claim 1 there is no explicit antecedent for "*the nucleic acid molecules* or for "*the immobilized nucleic acid molecules*" at lines 5 and 6 respectively. The description in general relates to "coded solid or semi-solid nucleic acid carriers" as stated at page 1 line 8 and if the field of the invention is confined to this then there is perhaps an implicit antecedent and the lack of clarity caused by the italicised words above can be resolved. On the other hand page 7 line 3 states that the invention provides " a carrier for nucleic acid, *or other molecules*" (emphasis added); if the carriers are not intended to be limited to carrying nucleic acid molecules then the lack of clarity cannot be so resolved.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

WO 1997/014028 discloses multiplexed diagnostic and genetic analyses of enzymes, DNA fragments, antibodies, and other biomolecules. The method comprises constructing an appropriately-labelled beadset, exposing the beadset to a clinical sample, and analysing the combined sample/beadset by flow cytometry. The flow cytometry measurements are used to classify beads within an exposed beadset to allow generation of textual explanations based on the accumulated data obtained during real-time analysis. The method allows the simultaneous, automated detection and interpretation of multiple biomolecules or DNA sequences in real-time. Again, the citation does not disclose or suggest the sorting step and so the present claims must be considered novel and inventive over this citation.

Proc. Natl. Acad. Sci. Vol 90, pp. 10700-10704, discloses a means for specifying the identity of each member of a library of molecules synthesised from both natural and unnatural chemical building blocks and the use of flow cytometry instrumentation for facile isolation of individual beads. It discloses the preparation of a library of peptide sequences by the combinatorial coupling of amino acid building blocks. Many copies of a single peptide sequence are covalently attached to each bead, to which additionally attached are copies of the single-stranded oligonucleotide that encodes that peptide. The oligonucleotide tags are synthesised through a parallel, combinatorial procedure that effectively records the process by which the peptide sequence was assembled. The collection of beads is screened using a fluorescence-activated cell-sorting instrument to isolate beads to which the antibody is tightly bound and the oligonucleotide identifiers attached to individual sorted beads are amplified by PCR. The present claims must be considered novel and inventive over this citation as the citation does not disclose or suggest the sorting step to provide a plurality of carriers including a population having detectably distinct code molecules.

All claims meet the criterion of being industrially applicable.

CLAIMS:

1. A method for producing a plurality of carriers including a population of carriers having detectably distinct code molecules, said method comprising:

(i) preparing a plurality of carriers having different code molecules wherein each code molecule is associated with a respective carrier;

(ii) subjecting the nucleic acid molecules to nucleic acid-based reactions to enable incorporation of detectable labels into the immobilized nucleic acid molecules;

(iii) identifying carriers having distinctive code molecules that are detectably and/or quantifiably deciperable or resolvable by the detection/quantification means;

(iv) identifying carriers having similar to non-distinctive code molecules; and

(v) sorting carriers having distinctive code molecules from the carriers having non-distinctive code molecules to thereby provide a plurality of carriers including a population having detectably distinct code molecules.

2. The method of Claim 1 wherein said nucleic acid molecules detectable label is attached via hybridization of a labeled primer or probe, optionally followed by amplification from said primer or probe.

3. The method of Claim 1 or 2 wherein said detectable label is a fluorescent label.

4. The method of any one of Claims 1 to 3 wherein sorting of said fluorescently labeled carriers according to the fluorescent label is performed using flow cytometry.

5. The method of any one of Claims 1 to 4 wherein identification of said carriers having said distinctive code molecule is performed using mass spectroscopy.

6. The method of Claim 1 wherein said molecule of each carrier is identified by an indirect or direct method for determining said nucleotide sequence of the nucleic acid molecule.

7. The method of Claim 6 wherein said nucleotide sequence is determined using an assay selected from the group consisting of amplification of the sequence or part thereof, in-vitro transcription, restriction fragment length determination, automated or manual sequencing.

8. A carrier produced according to the method of any one of Claims 1 to 7, said carrier comprising a solid or semi-solid support which is labeled with an identifiable code molecule that permits the differentiation of one such labelled carrier from another carrier in a heterogeneous population of said carriers.

9. The carrier of Claim 8 wherein said immobilized molecule is a known nucleic acid molecule.

10. The carrier of any one of Claims 8 or 9 wherein said code molecule is a peptide, that can be distinguished on the basis of molecular mass.

11. The carrier of any one of Claims 8 to 10 wherein said carrier further comprises a chemical linking moiety which is capable of forming a chemical bond with a nucleic acid molecule.

12. The carrier of Claim 11 wherein said chemical moiety comprises a thiol, carboxyl or amine group.

13. A nucleic acid molecule bound to or otherwise attached to said carrier of any one of Claims 8 to 11.

14. The carrier of Claim 8 wherein said immobilized molecule is a non-nucleic acid molecule and said code molecule is a nucleic acid which can be distinguished on the basis of nucleotide sequence.

15. The carrier of Claim 13 wherein said immobilized molecule is a putative protein-binding molecule.

16. The carrier of Claim 14 or 15 wherein said nucleic acid molecule is attached via a chemical linking moiety.

17. The carrier of Claim 16 wherein said chemical moiety comprises a thiol, carboxyl or amine group.

18. The carrier of any one of Claims 8 to 12 and 14 to 17 wherein said code or immobilized nucleic acid molecule is attached by a covalent bond between a chemical moiety on said surface of said carrier and a chemical moiety conjugated to said nucleic acid code molecule via a carbon atom spacer, having a structure mCn wherein n is the number of carbon atoms and is from 1 to about 100 and m is number of repeats of said Cn molecule and is from about 1 to about 10.

19. The carrier of any one of Claims 8 to 12 and 14 to 18 wherein either said code or said immobilized nucleic acid code molecule may be transcribed and/or comprises an RNA polymerase promoter sequence or functional fragment thereof.

20. The carrier of Claim 19 wherein the RNA polymerase promoter sequence is an SP6 RNA polymerase promoter sequence of functional fragment, homolog, analog, derivative thereof.

21. The carrier of any one of Claims 8 to 12 and 14 to 20 wherein said solid or semi-solid support is a microparticle.

22. A population of carriers of any one of Claims 8 to 12 and 14 to 20 wherein said population comprises one or more distinct classes of carrier on the basis of the attached code molecule.

23. A method for simultaneously detecting nucleotide polymorphisms in two or more subjects, said method comprising: amplifying or otherwise isolating a potentially polymorphic genetic sequence from two or more subjects in a population; binding the resultant polynucleotides from each subject to a uniquely coded carrier of any one of Claims 8 to 12; competitively hybridizing one or more differentially labeled probes or primers to the carrier bound nucleic acid, wherein each probe or primer is specific for a polymorphic variant; optionally performing an amplification reaction primed from the bound primer(s) or probe; sorting the population of carriers according to the bound label; and identifying a particular carrier present in each distinct labelled group on the basis of the molecular mass of the code molecule; thereby associating particular subjects with a particular polymorphic sequence variant.

24. A method for identifying small molecule ligands of a protein, said method comprising: producing or acquiring a library of putative ligands of the protein of interest; attaching each member of the library to a differentially coded carrier of any one of Claims 14 to 20; contacting the population of carriers with a labeled protein; sorting of the population by presence or absence of the bound label; and identification of the carriers that bind the subject protein by elucidation of the nucleotide sequence of the nucleic acid code; and thereby identification of a ligand of the protein by association of a library member with a particular code.

25. The method of Claim 23 or 24 wherein said carrier comprises a microsphere.

26. The method of any one of Claims 23 to 25 wherein said label is a fluorescent label.

27. The method of any one of Claims 23 to 26 wherein said carriers are sorted using flow cytometry and/or Fluorescence-Assisted Cell Sorting (FACS).

28. A kit comprising one or more coded carriers of any one of Claims 8 to 12 and 13 to 22 in compartmental form, said kit comprising one or more compartments containing one or more coded carriers, and optionally one or more compartments containing other reagents necessary for the use of said coded carriers, together with instructions for the use of said carriers.

29. A computer program product for assessing the codes on individual or groups of coded carriers according to any one of Claims 8 to 22, the product comprising:

(i) code that receives as input values, the code associated with a carrier;

(ii) code that compares said carrier code to provide assessment of the identity of carriers from a reference database; and

(iii) a computer readable medium that stores the codes.

30. A computer for assessing codes on carriers according to Claims 8 to 22, wherein said computer comprises:-

(i) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise values for the identity of codes on carriers;

(ii) a working memory for storing instructions for processing said machine-readable data;

(iii) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine readable data to compare said values to provide an assessment of the identity of codes from a reference database; and

(iv) an output hardware coupled to said central processing unit, for receiving the results of the identity of the codes.